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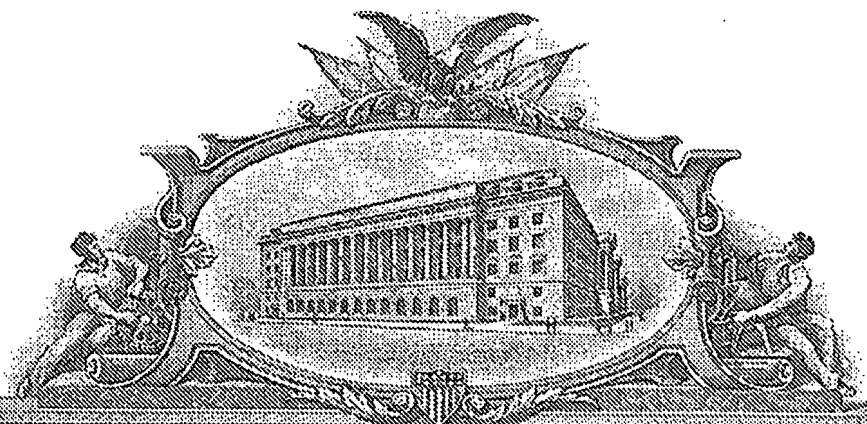
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February 16, 2005

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APPLICATION NUMBER: 60/531,168

FILING DATE: *January 20, 2004*

RELATED PCT APPLICATION NUMBER: *PCT/US05/02017*



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for Intellectual Property
and Director of the United States
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121903

16623 U.S. PTO

PTO/SB/16 (06-03)

Approved for use through 07/31/2003. OMB 0651-0032

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PROVISIONAL APPLICATION FOR PATENT COVER SHEET

This is a request for filing a PROVISIONAL APPLICATION FOR PATENT under 37 CFR 1.53(c).

Express Mail Label No. EV 117250566 US

16018 U.S. PTO
60/531168

INVENTOR(S)					
Given Name (first and middle [if any])		Family Name or Surname		Residence (City and either State or Foreign Country)	
Franklin W.		Okumu		San Diego, CA, USA	
Additional inventors are being named on the <u>1</u> separately numbered sheets attached hereto					
TITLE OF THE INVENTION (500 characters max)					
Treatment of Degenerative Cartilage Conditions in a Mammal with Glycosidase Inhibitors.					
Direct all correspondence to: CORRESPONDENCE ADDRESS					
<input checked="" type="checkbox"/> Customer Number:					
OR		32301			
<input type="checkbox"/> Firm or Individual Name		PATENT TRADEMARK OFFICE			
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ENCLOSED APPLICATION PARTS (check all that apply)					
<input checked="" type="checkbox"/> Specification Number of Pages <u>21</u>		<input type="checkbox"/> CD(s), Number _____			
<input type="checkbox"/> Drawing(s) Number of Sheets _____		<input type="checkbox"/> Other (specify) _____			
<input type="checkbox"/> Application Date Sheet. See 37 CFR 1.76					
METHOD OF PAYMENT OF FILING FEES FOR THIS PROVISIONAL APPLICATION FOR PATENT					
<input checked="" type="checkbox"/> Applicant claims small entity status. See 37 CFR 1.27.				FILING FEE Amount (\$)	
<input type="checkbox"/> A check or money order is enclosed to cover the filing fees.				<div>\$ 80.00</div>	
<input checked="" type="checkbox"/> The Director is hereby authorized to charge filing fees or credit any overpayment to Deposit Account Number: <u>502235</u>					
<input type="checkbox"/> Payment by credit card. Form PTO-2038 is attached.					
The invention was made by an agency of the United States Government or under a contract with an agency of the United States Government.					
<input checked="" type="checkbox"/> No.					
<input type="checkbox"/> Yes, the name of the U.S. Government agency and the Government contract number are: _____					

[Page 1 of 2]

Respectfully submitted

SIGNATURE

TYPED or PRINTED NAME

TELEPHONE 858-450-0099

Thomas E. Jurgensen, Esq.

Date December 19, 2003

REGISTRATION NO. 34,195

(if appropriate)

Docket Number: 8024-014-PR

USE ONLY FOR FILING A PROVISIONAL APPLICATION FOR PATENT

This collection of information is required by 37 CFR 1.51. The information is required to obtain or retain a benefit by the public which is to file (and by the USPTO to process) an application. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.14. This collection is estimated to take 8 hours to complete, including gathering, preparing, and submitting the completed application form to the USPTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden, should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, U.S. Department of Commerce, P.O. Box 1450, Alexandria, VA 22313-1450. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. SEND TO: Mail Stop Provisional Application, Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450.

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Additional Pag

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Docket Number 8024-014-PR

INVENTOR(S)/APPLICANT(S)		
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[Page 2 of 2]

Number 1 of 1

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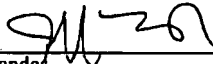
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December 19, 2003

Certificate of Express Mail Mailing

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Jeff Lande

12/19/03
Date of Deposit

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**Re: TREATMENT OF DEGENERATIVE CARTILAGE CONDITIONS IN A MAMMAL WITH
GLYCOSIDASE INHIBITORS**
Our Docket No.: 8024-014-PR

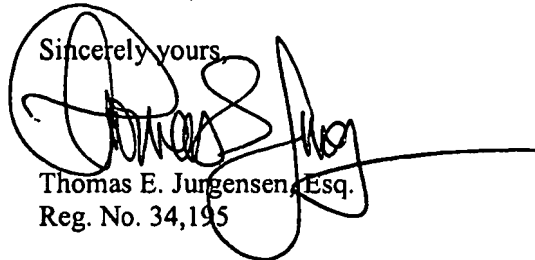
Dear Sir or Madam:

Enclosed please find the following documents related to the above-identified matter:

1. Provisional Application for Patent Cover Sheet (PTO/SB/16);
2. Fee Transmittal (PTO/SB/17);
3. Application Document (21 pages); and
4. Self-addressed, stamped postcard.

The self-addressed, stamped postcard has been included for your convenience. After confirming receipt of these documents please return the postcard to us at your earliest convenience. Should you have any questions, please do not hesitate contacting me.

Sincerely yours,


Thomas E. Jurgensen, Esq.
Reg. No. 34,195

Enclosures

121503

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U.S. PTO

PTO/SB/17 (05-03)

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**FEE TRANSMITTAL
for FY 2003**

Effective 01/01/2003. Patent fees are subject to annual revision.

☒ Applicant claims small entity status. See 37 CFR 1.27

TOTAL AMOUNT OF PAYMENT (\$) 80.00

Complete If Known

Application Number	
Filing Date	December 19, 2003
First Named Inventor	Okumu, Franklin W.
Examiner Name	
Art Unit	
Attorney Docket No.	8024-014-PR

METHOD OF PAYMENT (check all that apply)☐ Check ☐ Credit card ☐ Money Order ☐ Other ☐ None☒ Deposit Account:Deposit Account Number: 502235
Deposit Account Name: BKF Jurgensen

The Director is authorized to: (check all that apply)

☒ Charge fee(s) indicated below ☐ Credit any overpayments☐ Charge any additional fee(s) during the pendency of this application☐ Charge fee(s) indicated below, except for the filing fee to the above-identified deposit account.**FEE CALCULATION****1. BASIC FILING FEE**

Large Entity Fee Code (\$)	Small Entity Fee Code (\$)	Fee Description	Fee Paid
1001 750	2001 375	Utility filing fee	
1002 330	2002 165	Design filing fee	
1003 520	2003 260	Plant filing fee	
1004 750	2004 375	Reissue filing fee	
1005 160	2005 80	Provisional filing fee	80.00

SUBTOTAL (1) (\$) 80.00

2. EXTRA CLAIM FEES FOR UTILITY AND REISSUE

Total Claims	Extra Claims	Fee from below	Fee Paid
Independent	-20** =	X	
Multiple Dependent	-3** =	X	

Large Entity Fee Code (\$)	Small Entity Fee Code (\$)	Fee Description
1202 18	2202 9	Claims in excess of 20
1201 84	2201 42	Independent claims in excess of 3
1203 280	2203 140	Multiple dependent claim, if not paid
1204 84	2204 42	** Reissue independent claims over original patent
1205 18	2205 9	** Reissue claims in excess of 20 and over original patent

SUBTOTAL (2) (\$)

**or number previously paid, if greater; For Reissues, see above

FEE CALCULATION (continued)**3. ADDITIONAL FEES**

Large Entity Fee Code (\$)	Small Entity Fee Code (\$)	Fee Description	Fee Paid
1051 130	2051 65	Surcharge - late filing fee or oath	
1052 50	2052 25	Surcharge - late provisional filing fee or cover sheet	
1053 130	1053 130	Non-English specification	
1812 2,520	1812 2,520	For filing a request for ex parte reexamination	
1804 920*	1804 920*	Requesting publication of SIR prior to Examiner action	
1805 1,840*	1805 1,840*	Requesting publication of SIR after Examiner action	
1251 110	2251 55	Extension for reply within first month	
1252 410	2252 205	Extension for reply within second month	
1253 930	2253 465	Extension for reply within third month	
1254 1,450	2254 725	Extension for reply within fourth month	
1255 1,970	2255 985	Extension for reply within fifth month	
1401 320	2401 160	Notice of Appeal	
1402 320	2402 160	Filing a brief in support of an appeal	
1403 280	2403 140	Request for oral hearing	
1451 1,510	1451 1,510	Petition to institute a public use proceeding	
1452 110	2452 55	Petition to revive - unavoidable	
1453 1,300	2453 650	Petition to revive - unintentional	
1501 1,300	2501 650	Utility issue fee (or reissue)	
1502 470	2502 235	Design issue fee	
1503 630	2503 315	Plant issue fee	
1460 130	1460 130	Petitions to the Commissioner	
1807 50	1807 50	Processing fee under 37 CFR 1.17(q)	
1808 180	1808 180	Submission of Information Disclosure Stmt	
8021 40	8021 40	Recording each patent assignment per property (times number of properties)	
1809 750	2809 375	Filing a submission after final rejection (37 CFR 1.129(a))	
1810 750	2810 375	For each additional invention to be examined (37 CFR 1.129(b))	
1801 750	2801 375	Request for Continued Examination (RCE)	
1802 900	1802 900	Request for expedited examination of a design application	

Other fee (specify)

*Reduced by Basic Filing Fee Paid

SUBTOTAL (3) (\$)

SUBMITTED BY

Name (Print/Type)	Thomas, E Jurgensen, Esq	Registration No. (Attorney/Agent)	34,195	Telephone	858-450-0099
Signature		Date	12/19/2003		

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Date: December 19, 2003 Express Mail Label No.: EV 117250566 US

Inventor(s): Franklin W. Okumu,
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Attorney's Docket No.: 8024-014-PR

**TREATMENT OF DEGENERATIVE CARTILAGE CONDITIONS IN A MAMMAL
WITH GLYCOSIDASE INHIBITORS**

TECHNICAL FIELD:

The present invention relates to treating, preventing, and lessening the severity of conditions selected from the group consisting of synovitis, subchondral bone edema, and cartilage degradation with administration of glycosidase inhibitors.

BACKGROUND OF THE INVENTION:

Synovitis, subchondral bone edema, progressive cartilage degradation and other similar conditions are separate and distinct conditions of the joints due to, among other things, physical injuries. These conditions may also be associated with osteoarthritis (OA) or rheumatoid arthritis (RA) (Ayrar et. al.

Rheumatology, Vol 35, 14-17; McAlindon, Best Pract Res Clin Rheumatol 1999; 13(2):329-44). For example, they may appear as secondary conditions to OA or RA or on their own due to other injuries. Osteoarthritis is the most common joint disease, currently affecting approximately 40 million Americans. This number is
5 expected to increase to 60 million within the next 20 years. Diverse risk factors contribute to the multifactorial etiology of OA. The central pathogenetic mechanism in OA is an aberrant cartilage matrix remodeling process with loss of cartilage cells and matrix, resulting in biomechanical joint failure and inflammation.

10 Common in these conditions is an erosion of the cartilage. Cartilage erosion results from the over-catabolism of glycosaminoglycans (GAGs) of the proteoglycan (PG)-hyaluronate complex, which comprises the bulk of cartilage tissue. It is known that patients with arthritis, for example, have an abnormal increase of beta-N-acetylhexosaminidase activity in the synovial fluid (O. Kida, J.
15 Japan Orthop. Assoc. 1968, 42(6), 4010; R.W. Stephen, et al., Biochim. Biophys. Acta 1975, 399(1), 101; and J.J. Steinberg, et al., Biochim. Biophys. Acta 1983, 757(1), 47). In rheumatoid arthritis, for example, the dominant glycosidases are the hexosaminidases, such as beta-D-N-acetylglucosaminidase and beta-D-N-acetyl-galactosiminidase. These hexosaminidases, acting either alone or in
20 combination with other glycosidases such as beta-D-glucuronidase, were shown to be directly involved in depleting GAGs from cartilage (Z. Ortutay, et al., Arthritis Rheum. 2003, 48(8), 2163). Thus, Applicants have designed and synthesized a specific hexosaminidase inhibitor that was extremely potent,

having a K_i of 24 nM against hexosaminidase and thereby preventing cytokine-induced loss of GAGs in cultured chondrocytes (J. Liu, et al., Chem. Biol. 2001, 8(7), 701). Further studies revealed that this inhibitor could provide a chondroprotective benefit in an osteoarthritis animal model (*vide infra*).

5 Unfortunately, the prior art does not provide for an effective means of treating, preventing, and lessening the severity of synovitis, subchondral bone edema, and cartilage degradation. Accordingly, there remains a great need for methods to treat, prevent, and lessen the severity of these conditions, which overcomes the shortcomings of the prior art.

10 **BRIEF SUMMARY OF THE INVENTION:**

Applicants have determined that inhibition of glycosidases in the synovial fluid has great utility as a novel chondroprotective approach in treating diseases associated with cartilage degradation. Administration of said inhibitors against these targets are useful therapeutic interventions for treating synovitis,
15 subchondral bone edema, cartilage degradation, and other similar conditions. Therefore, inhibition of the glycosidases in synovial fluid has great utility as a novel approach to chondroprotection.

One embodiment of the present invention relates to using glycosidase inhibition to address the inflammatory and cartilage-degrading conditions of joint
20 diseases. Said joint diseases include, but are not limited to synovitis, subchondral bone edema, cartilage degradation, and other similar conditions. According this embodiment, inhibitors of glycosidases such as hexosaminidases

or glucuronidases can be utilized as chondroprotective agents that interfere with the breakdown of the cartilage matrix of the joint.

In another preferred embodiment of the present invention, an inhibitor of hexosaminidase, demonstrated unexpected chondroprotective effects in mammals. These chondroprotective properties give rationale to utilizing a glycosidase inhibitor(s) as a therapeutic approach for treating, for example, osteoarthritis (OA), or rheumatoid arthritis (RA).

Another preferred embodiment of the present invention contemplates the utilization of a single glycosidase inhibitor in combination with other glycosidase inhibitor(s) and/or another anti-inflammatory drug(s) or aminosugars for treating arthritis.

A preferred embodiment of the present invention relates to methods of treating, preventing, and lessening the severity of synovitis, subchondral bone edema, and cartilage degradation by administering to a patient a therapeutically effective amount of a glycosidase inhibitor, such as a hexosaminidase inhibitor, or glucuronidase inhibitor or a combination thereof. A therapeutically effective amount of the inhibitors can be administered to a patient by any means well known in the art, including, but not limited to orally, intravascularly, intramuscularly, topically or intra-articularly. A therapeutically effective amount of such inhibitors may also be administered intra-articularly in a matrix as a controlled release or sustained release formulation.

Another preferred embodiment of the present invention, the invention relates to a method including administering to a patient a composition containing

a therapeutically effective amount of a glycosidase inhibitor (such as a hexosaminidase inhibitor(s) or a glucuronidase inhibitor(s) or a combination thereof), either alone or in combination with an existing anti-inflammatory drug or other therapeutic molecule. Methods of administering formulations of the present invention include, but are not limited to, intravascular, intra-articular, topical, oral, and intra-muscular methods.

In one embodiment of the method, a combination of glycoside inhibitors having a specific activity or a variety of activities against hexosaminidase, glucuronidase or other endo- and exoglycosidases may also be used to achieve a chondroprotective effect in the joint.

BRIEF DESCRIPTION OF THE DRAWINGS:

Figure 1 shows the Femur Lesion Grades 8 weeks after anterior cruciate ligament transection (ACLT) surgery for animals treated with saline or a hexosaminidase inhibitor ((2R,3R,4R,5R)-N-methyl-2-(acetamidomethyl)-3,4-dihydroxy-5-(hydroxymethyl)pyrrolidine). Error bars represent one standard deviation.

Figure 2 shows the Tibial Lesion Grades eight (8) weeks after ACLT surgery for animals treated with saline or with the specific hexosaminidase inhibitor ((2R,3R,4R,5R)-N-methyl-2-(acetamidomethyl)-3,4-dihydroxy-5-(hydroxymethyl)pyrrolidine). Error bars represent one standard deviation.

Figure 3 shows the Joint Swelling Grading 8 weeks after ACLT surgery for animals treated with saline or with the specific hexosaminidase inhibitor ((2R,3R,4R,5R)-N-methyl-2-(acetamidomethyl)-3,4-dihydroxy-5-(hydroxymethyl)pyrrolidine). Error bars represent one standard deviation.

Figure 4 shows the Synovial Fluid Grading 8 weeks after ACLT surgery for animals treated with saline or with the specific hexosaminidase inhibitor ((2R,3R,4R,5R)-N-methyl-2-(acetamidomethyl)-3,4-dihydroxy-5-(hydroxymethyl)pyrrolidine). Error bars represent one standard deviation.

Figure 5 shows the effect the the specific hexosaminidase inhibitor ((2R,3R,4R,5R)-N-methyl-2-(acetamidomethyl)-3,4-dihydroxy-5-(hydroxymethyl)pyrrolidine) has on the prevention of IL-1.beta.-induced sGAG loss. Error bars represent one standard deviation.

DETAILED DESCRIPTION OF THE INVENTION:

Applicants have determined that inhibition of glycosidases in the synovial fluid has great utility as a novel chondroprotective approach in treating diseases associated with cartilage degradation. Administration of said inhibitors against these targets are useful therapeutic interventions for treating synovitis, subchondral bone edema, cartilage degradation, and other similar conditions.

Therefore, inhibition of the glycosidases in synovial fluid has great utility as a novel approach to chondroprotection.

For example, accelerated loss of proteoglycans and glycosaminoglycans is a hallmark of osteoarthritic cartilage. Increased catabolism of proteoglycans and glycosaminoglycans compromises both the functional and structural integrity of the cartilage matrix and eventually renders the tissue incapable of resisting the compressive loads applied during joint movement (Inerot, S., et al., *Biochem. J.*, 1978, 169(1), 143). Over time, this process leads to irreversible cartilage degeneration. Loss of articular proteoglycans in established joint disease could be more significant than the collagen loss (Mankin, H.J. et al., 1970 *J. Bone Joint Surg. Am.*, 52(3), 424). In addition to quantitative changes, affected cartilage also undergoes certain qualitative changes. Among these changes are a disproportionately increased ratio of chondroitin 4-sulfate to chondroitin 6-sulfate; a decreased ratio of keratan sulfate to chondroitin sulfate (Mankin, H.J., et al., 1971, *J. Clin. Invest.* 50(8): 1712); and a decreased sulfation of the terminal residues in chondroitin and dermatan sulfate chains (Plaas, A.H., et al., 1998, *J. Biol. Chem.* 273(20), 12642).

Degradation of the cartilage matrix is a multifactorial process involving the degradation of glycosaminoglycans by the glycosidases and involving the action of several metalloproteinases, such as collagenases, stromelysins, aggrecanases, and cysteine proteases such as cathepsins. (Winchester, B.G., 1996 *Subcell.Biochem.* 27, 191; Kresse, H., et al. 1987, *Adv. Enzymol. Relat. Areas Mol. Biol.*, 60, 217).

Glycosidases are produced by chondrocytes and possess an enzymatic activity towards several glycosaminoglycans. Hexosaminidase is a dominant glycosaminoglycan-degrading glycosidase released by the chondrocytes into the extracellular compartment, and it is the dominant glycosidase in synovial fluid of
5 patients with osteoarthritis (Shikhman, A. et al., 2000 Arthritis Rheum. 43, 1307).

Hexosaminidase belongs to the group of lysosomal hydrolases. Hexosaminidase catalyzes the hydrolysis of terminal, non-reducing N-acetyl-.beta.-D-glucosamine and N-acetyl-.beta.-D-galactosamine residues in glycoproteins, G.sub.M2-gangliosides, and glycosaminoglycans, including
10 chondroitin 4-sulfate, chondroitin 6-sulfate, hyaluronic acid, keratan sulfate and dermatan sulfate (Winchester, B.G. 1996 ibid.).

The present invention provides examples to teach how the utilization of a glycosidase inhibitor (such as a hexosaminidase inhibitor) can produce a desired chondroprotective effect in a pathologic condition of cartilage such as synovitis,
15 subchondral bone edema and cartilage degradation. This approach represents a novel means to prevent cartilage matrix glycosaminoglycan degradation and a new strategy for treating osteoarthritis, rheumatoid arthritis, inflammatory joint diseases, traumatic joint diseases and related pathologic conditions.

20 **Abbreviations and Terms:**

In accordance with the present invention and as used herein, the following terms and abbreviations are defined with the following meanings, unless explicitly stated otherwise. These explanations are intended to be exemplary only, and

are not intended to limit the terms as they are described or referred to throughout the specification. These explanations include any additional aspects and/or examples of the terms as described and claimed herein.

- 5 GAGs = glycosaminoglycans;
- HA = hyaluronic acid;
- IL-1.beta. = interleukin-1.beta.;
- IL-6 = interleukin-6;
- OA = osteoarthritis;
- 10 RA = rheumatoid arthritis;
- sGAG = sulfate-containing form of glycosaminoglycan

The term "therapeutically effective amount" refers to the amount of a biologically active substance necessary to induce a desired pharmacological
15 effect. The amount can vary greatly according to the effectiveness of a particular active substance; the age, weight, and response of the individual; as well as the nature and severity of the individual's symptoms. Accordingly, there is no upper or lower critical limitation with respect to the amount of the active substance. A therapeutically effective amount to be employed in the present invention can
20 readily be determined by those ordinarily skilled in the art.

The term "aminosugar" refers to any synthetic or naturally occurring sugar wherein one or more carbon atoms are substituted with an amino group (-NR.sup.1R.sup.2). Such substitution may occur without regard to orientation or

configuration of any asymmetric carbons present in the sugar. Unless stated otherwise, the term "aminosugar" refers to either anomer (alpha or .beta.) of a cyclic or open chain amino sugar. Aminosugars may be N-substituted with alkyl or acyl group, where one hydrogen atom of a pendant amino group is replaced
5 by an alkyl or acyl moiety (-COR where R = lower alkyl).

The term "arthritis" refers to any particular disease characterized by joint inflammation, although the etiology of the inflammation may differ in various conditions. Relatively common arthritic diseases include rheumatoid arthritis, juvenile arthritis, ankylosing spondylitis, psoriatic arthritis, traumatic arthritis and
10 osteoarthritis. Osteoarthritis is also referred to as "degenerative joint diseases."

The terms "articular cartilage" or "cartilage" refer to a substance that covers ends of bones and forms the joint surfaces. Cartilage can withstand compressive forces and creates a low friction surface for a joint to glide on. Articular cartilage comprises chondrocytes and a substrate comprising proteins
15 and glycosaminoglycan polysaccharides.

The term "cartilage degradation" refers to degradation in the tissues comprising cartilage.

The term "chondrocyte" refers to cells found within articular cartilage. Chondrocytes produce collagen, a gelatinous protein, and proteoglycans, which
20 are glycosaminoglycans linked to proteins (also called mucopolysaccharides).

The term "less severe" refers to a particular grade in cartilage degradation of patient. Preferably, less severe grade is in the range of Grade 1 to Grade 3. More preferably, less severe grade is in the range of Grade 1 to Grade 2.

The term glucuronic acid refers to a derivative of D-glucose with a COOH group at the C-5 position that is a major component of GAGs.

The term glucuronidase refers to an enzyme which releases a glucuronic acid residue from a substrate such as GAG.

5 The term "glycosaminoglycan" refers to long heteropolysaccharide molecules containing repeating disaccharide units. The disaccharide units may comprise normal modified amino sugars: D-N-acetylgalactosamine or D-GlcNAc and an uronic acid such as D-glucuronate or L-iduronate. Among other functions, GAGs serve as a lubricating fluid in the joints. Specific GAGs of
10 physiological significance are hyaluronic acid, dermatan sulfate, chondroitin sulfate, heparin, heparan sulfate, and keratan sulfate.

The term "hyaluronic acid" refers to a naturally occurring linear polysaccharide formed by repeating disaccharide units consisting of D-glucuronic acid .beta.(1-3) N-acetyl-D-glucosamine linked by .beta.(1-4) glycosidic linkages.

15 The term "IL-1.beta." refers to interleukin-1.beta., an immunomodulator that mediates a wide range of immune and inflammatory responses, including the activation of B- and T -cells.

The term "intra-articular" refers to a method of delivering a drug directly to a joint. Traditional routes of drug delivery, such as for example, oral, intravenous
20 or intramuscular administration, depend upon vascular perfusion of the synovium to carry the drug to the joint. This is inefficient because transsynovial transfer of small molecules from the synovial capillaries to the joint space generally occurs by passive diffusion, which becomes less efficient with increasing size of the

target molecule. Thus, the access of directing molecules, for example, glucosamine, to the joint space is substantially restricted. Intra-articular injection or perfusion of drugs circumvents such limitations.

The term "sustained release" refers to the time period during which a drug
5 is released for availability, or otherwise becomes available for physiological uptake. Periods of sustained release may be preceded by an induction period, during which little or no drug is released, or may be biphasic, comprising an initial time period during which some drug is released, and a second time period during which additional drug is released.

10 In contrast, the term "continuous release" is used solely to describe a release profile that appears to be monophasic, having a smooth-curved time profile of release. Those of skill in the art will appreciate that the release profile may actually correspond to an exponential or logarithmic time-release profile.

15 Examples:

The present invention will be described in more detail by the following examples, which should not be construed as limiting the present invention.

Example 1

20 Effect of Continuous Infusion of a Hexosaminidase Inhibitor in an
Osteoarthritis Animal Model.

The model used in this study is the transection of the anterior cruciate ligament (ACL) in the rabbit knee. ACL transection (ACLT) causes joint instability and subsequent development of degradative and osteoarthritis-like changes.

5 Female New Zealand White rabbits, 3.0-3.5 kg, were used and were randomly allocated into groups of 8 rabbits. Group A was the saline-treated control group; Group B was treated with 30 mM of the hexosaminidase inhibitor (2R,3R,4R,5R)-N-methyl-2-(acetamidomethyl)-3,4-dihydroxy-5-(hydroxymethyl)pyrrolidine. All the compounds were delivered by a 2ml Alzet
10 osmotic pump (Alzet 2ML4, Alza, USA). The delivery rate from the pump was 2.5 microliters/hour. All rabbits are received ACLT surgery on the right knee.

All rabbits were anesthetized by an intramuscular injection of ketamine (35 mg/kg) and acepromazine (2.5 mg/kg). Knees were shaved and disinfected with Antibex (Vetoquinol S.A.) solution. A medial parapatellar incision was made on
15 the skin and a medial arthrotomy was performed. The patella was dislocated laterally and the knee was placed in full flexion. The ACL was visualized and transected with a #15 blade. Complete transection was confirmed by a manual anterior drawer test. The joint was irrigated with sterile saline then closed. Before the closure of knee joint, an Alzet pump prefilled with compounds (either
20 with saline or with the hexosaminidase inhibitor (2R,3R,4R,5R)-N-methyl-2-(acetamidomethyl)-3,4-dihydroxy-5-(hydroxymethyl)pyrrolidine) was implanted subcutaneously in the lower right abdomen of rabbits. The pump was connected by polyethylene tubing (ID: 0.58 mm), which was inserted into the right knee joint

with its tip resting in the synovial space. The joint capsule was closed with a running suture and the tubing connecting the pump was also fixed into the tissue. The skin was closed with interrupted sutures. The Alzet pumps were replaced with fresh units at the end of the fourth week after the operation.

5 The animals were sacrificed eight (8) weeks after ACLT. The gross morphological changes of both knees, including joint swelling and joint fluid, are evaluated. The occurrence, site and severity of lesions on the surface of the femurs and tibia were determined during observations under a dissecting microscope using the following criteria: Grade 1 (Intact surface), Surface is
10 normal in appearance and does not retain Indian ink; Grade 2 (Minimal fibrillation), Surface retains India ink as elongated specks or light gray patches; Grade 3 (Overt fibrillation), Areas which are velvety in appearance and retain India ink as intense black patches; Grade 4 (Erosion), Loss of cartilage exposing the underlying bone.

15 The grading of the joint swelling is as following: 0 (normal); 1 (mild), inflammation and/or proliferation of the joint capsule; 2 (moderate), thickening of joint capsule and/or inflammation of the synovium; 3 (severe) abundant inflammation of the synovium, swelling of the menisci or ligaments (anterior or posterior cruciate ligaments). The grading of the joint fluid is as following:
20 (normal); 1 (mild) Fluid is greater than normal, but does not fill the knee joint; 2 (moderate) Fluid fills the knee joint, but does not pour out of the capsule as it is opened; 3 (severe) Fluid expands the knee joint and pours out as the capsule is opened.

Results in Example

Observations on the knee tissues revealed that treatment with the hexosaminidase inhibitor (2R,3R,4R,5R)-N-methyl-2-(acetamidomethyl)-3,4-dihydroxy-5-(hydroxymethyl)pyrrolidine resulted in consistently less chondrodegenerative pathology to the knee joint, compared to saline-treated animals.

Figures 1 and 2 show that treatment with the hexosaminidase inhibitor (2R,3R,4R,5R)-N-methyl-2-(acetamidomethyl)-3,4-dihydroxy-5-(hydroxymethyl)pyrrolidine leads to a statistically significant decrease in the severity of femur and tibia lesion scores, respectively, compared to saline-treated animals.

Figure 3 shows that treatment with (2R,3R,4R,5R)-N-methyl-2-(acetamidomethyl)-3,4-dihydroxy-5-(hydroxymethyl)pyrrolidine produces a trend towards decreased joint-swelling, compared to saline-treated animals.

Figure 4 shows that treatment with (2R,3R,4R,5R)-N-methyl-2-(acetamidomethyl)-3,4-dihydroxy-5-(hydroxymethyl)pyrrolidine results in normal levels of fluid in the knee joint. In contrast, there were increased effusions in the knees of the saline-treated animals. The scoring difference between the (2R,3R,4R,5R)-N-methyl-2-(acetamidomethyl)-3,4-dihydroxy-5-(hydroxymethyl)pyrrolidine group and the saline-treated group was statistically significant.

Example 2

Reduction of sGAG loss By Continuous Infusion of a Hexosaminidase Inhibitor

Female New Zealand White rabbits, 3.0-3.5 kg, were used and were
5 randomly allocated into groups of 8 rabbits. Under aseptic conditions, 2 ml Alzet
osmotic pumps (delivery flow rate: 10 microliter/h, (Alzet 2ML1, Alza, USA)) were
filled with IL-1.β. (1000 U/ml; R&D Systems, USA). Separate 2 ml Alzet
pumps were filled with 30 mM of the hexosaminidase inhibitor 2R,3R,4R,5R)-N-
methyl-2-(acetamidomethyl)-3,4-dihydroxy-5-(hydroxymethyl)pyrrolidine or with
10 saline. The Alzet pumps were implanted subcutaneously in the lower abdomen
of rabbits, and were connected by a polyethylene tubing (ID: 0.025 in) threaded
subcutaneously to the left knee joint with their tips resting in the synovial space.
The untreated contralateral knees of all animals served as negative controls. IL-
1.β. and (2R,3R,4R,5R)-N-methyl-2-(acetamidomethyl)-3,4-dihydroxy-5-
15 (hydroxymethyl)pyrrolidine were infused intra-articularly for 7 days. Immediately
before surgery, and for 3 consecutive days thereafter, Marbocyl, 6 mg/kg/day, is
administered (i.m.) to prevent infection. Animals were harvested on day 7.

Sulfated Glycosaminoglycan Assay

20 Rabbits were sacrificed by i.v. injection of pentobarbital. Both knee joints
were removed and the articular cartilages of lateral tibia plateaus were harvested
using a scalpel while visualized under a dissection microscopy. The cartilages
were weighed and stored at -80.°C. For analysis, the samples were thawed

just prior to the assay. sGAGs were extracted from the sample using freshly prepared papain (1 mg papain in 2 ml of 50 mM phosphate buffer, pH 6.8 (PBS), containing 1.0 M NaCl, 5 mM, cysteine-HCl and 1 mM EDTA). 1 mg cartilage was digested with 20 microliter PBS-papain solution while stirred at 60.deg.C for
5 24 hrs. After digestion, the samples were centrifuged at 10,000 rpm for 10 min, the supernatants then diluted 75 times with PBS-papain buffer then in the following assay.

Blyscan dye (Biocolor, USA) was added to each tube and vortexed. The solution was mixed at 90 rpm using a mechanical shaker for 30 minutes then
10 centrifuged at 20,000 x g. The pellets were reconstituted with 450 microliters of Blyscan dissociation reagent and vortexed for at least 3 min to fully dissolve the pellets. The absorbance of the sample was then observed at 656 nm using a spectrophotometer. Ultra pure water was used as blank. The absorbance of the blank was subtracted from each sample. Results are expressed as micrograms
15 of sGAG recovered per milligram wet weight of cartilage tissue.

The recoverable sGAG content in the cartilage of the left tibial plateau of untreated knees was about 46 micrograms per milligram of wet weight of tissue (Figure 5). Following infusion, the amount of recoverable sGAG in saline-treated knees was about 28% less than in the non- IL-1.beta.-treated control left knee
20 (33 .micro.g/mg of wet weight of left tibial plateau cartilage vs. 46 .micro.g/mg). In the (2R,3R,4R,5R)-N-methyl-2-(acetamidomethyl)-3,4-dihydroxy-5-(hydroxymethyl)pyrrolidine treated knees, the amount of recoverable sGAG was approximately 41 .micro.g/mg, a 24% increase over saline-treated knees.

CLAIMS:

We claim:

- 5 1. A method for curing, treating, preventing or lessening the severity of a condition selected from the group consisting of synovitis, subchondral bone edema and cartilage degradation by the administration of a sufficient quantity of at least one glycosidase inhibitor.
- 10 2. The method of claim 1, wherein said cure, treatment, prevention or lessening of the severity of said condition is by the administration of a sufficient quantity of at least one glycosidase inhibitor in a cocktail with a second therapeutic molecule selected from the group consisting of anti-inflammatory agents and aminosugars.
- 15 3. The method of claim 1, wherein said cure, treatment, prevention or lessening of the severity of said condition is by the administration of a sufficient quantity of a cocktail comprising at least one glycosidase inhibitor in a cocktail with an anti-inflammatory agent and an aminosugar.
- 20 4. The method of claim 1, wherein said glycosidase inhibitor is selected from the group consisting of, glycosidase inhibitors, non-iminocyclitol glycosidase inhibitors, hexosaminidase inhibitors and glucuronidase inhibitors.

5. The method of claim 1, wherein said glycosidase inhibitor has a specific activity against a glycosidase selected from the group comprising, glycosidases, hexosaminidases, glucuronidases, endoglycosidases and exoglycosidases.
- 5 6. The method of claim 1, wherein said condition results in osteoarthritis.
7. The method of claim 1, wherein said condition results in rheumatoid arthritis.
8. The method of claim 1, wherein said condition results in arthritis.
- 10 9. The method of claim 1, wherein said condition results in inflammatory joint disease.
10. The method of claim 1, wherein said condition results in traumatic joint disease.
- 15 11. The method of claim 1, wherein said administration is by a route selected from the group consisting of oral, intra-vascularly, intra-articularly, intra-muscularly and topically.
- 20 12. The method of claim 1, wherein said glycosidase inhibitor is formulated in a sustained or controlled release formulation.

13. The method of claim 1, wherein said glycosidase inhibitor is delivered to the disease site using a delivery device.

14. The method of claim 13, wherein said delivery device is an Alzet Pump

5

15. A method for curing, treating, preventing or lessening the severity of an inflammatory condition by the administration of a sufficient quantity of at least one glycosidase inhibitor.

10 16. The method of claim 15, wherein said administration is by a route selected from the group consisting of oral, intra-vascularly, intra-articularly, intra-muscularly and topically.

17. The method of claim 15, wherein said glycosidase inhibitor is formulated in a
15 sustained or controlled release formulation.

18. The method of claim 15, wherein said glycosidase inhibitor is delivered to the disease site by a delivery device(s).

20 19. The method of claim 18, wherein said delivery device is an Alzet pump.

ABSTRACT OF THE DISCLOSURE

This invention relates to treating, preventing, and lessening the severity of conditions selected from the group consisting of synovitis, subchondral bone
5 edema, and cartilage degradation with administration of glycosidase inhibitors.

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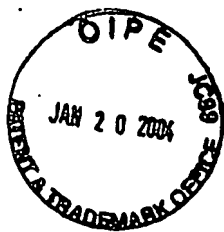
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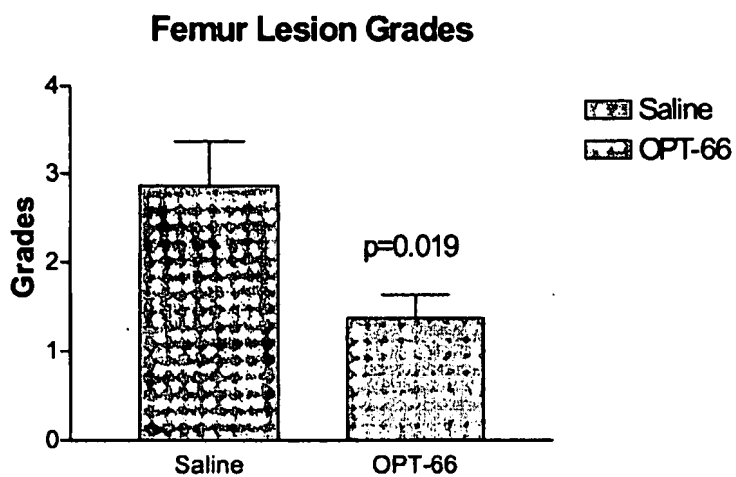
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Drawing Sheet 1 of 5

Figure 1

**Femur Lesion Grading
Hexosaminidase Inhibitor (OPT-66) vs. Saline**

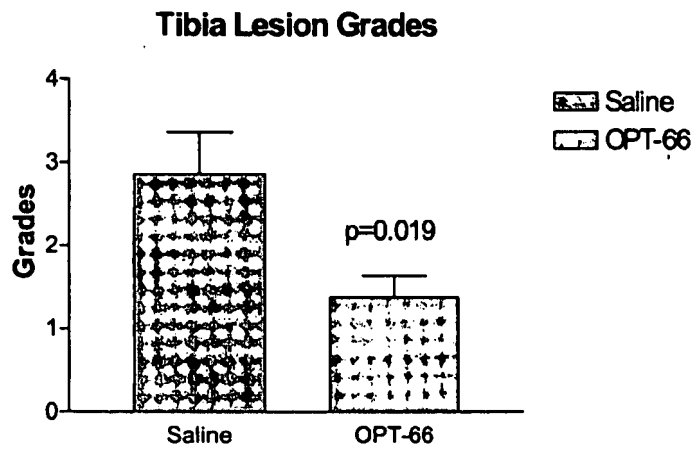




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Drawing Sheet 2 of 5

Figure 2
Tibia Lesion Grading:
Hexosaminidase Inhibitor (OPT-66) vs. Saline

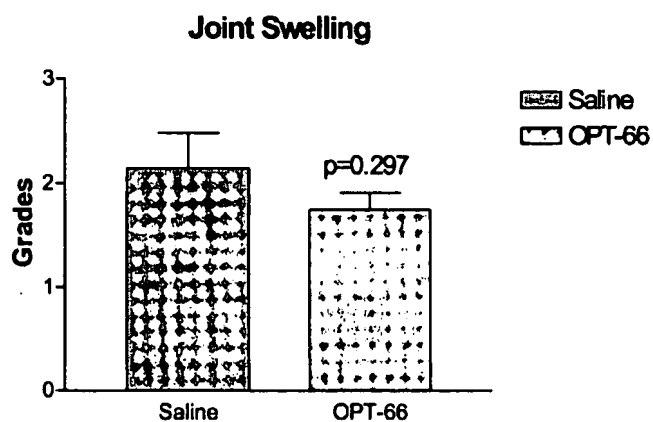




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Figure 3
Joint Swelling Grading:
Hexosaminidase Inhibitor (OPT-66) vs. Saline

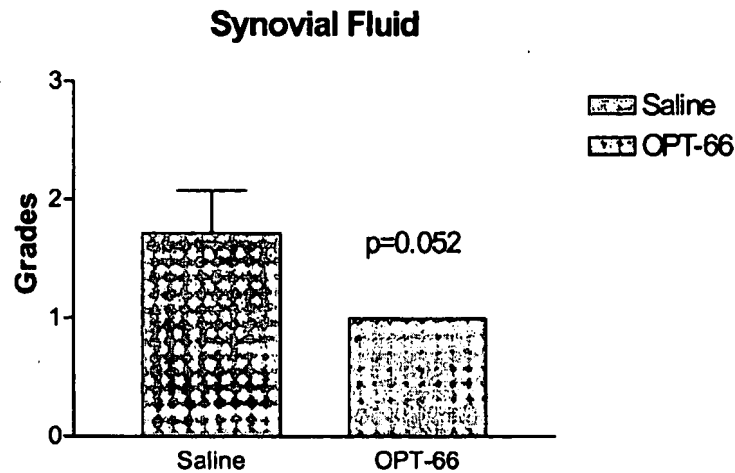




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Figure 4
Synovial Fluid Grading:
Hexosaminidase Inhibitor (OPT-66) vs. Saline





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IN A MAMMAL WITH GLYCOSIDASE INHIBITORS

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Drawing Sheet 5 of 5

Figure 5

Effect of Hexosaminidase Inhibitor (OPT-66)
On IL-1 β -Induced sGAG Loss

